

**Amendments to the Specification**

Please replace the paragraph beginning at page 19, line 25, with the following amended paragraph:

The partially purified enzyme preparation having  $\alpha$ -isomaltosyl glucosaccharide-forming activity, thus obtained, was dialyzed against 10 mM sodium phosphate buffer (pH 7.0) with 1 M ammonium sulfate. The dialyzed solution was centrifuged to remove impurities, and subjected to an affinity column chromatography using 500 ml of "SEPHACRYL HR S-200", a gel commercialized by Amersham Biosciences K. K., Tokyo, Japan. The enzyme was adsorbed on "SEPHACRYL HR S-200" gel and, when eluted with a linear gradient decreasing from 1 M to 0 M of ammonium sulfate, the enzymatic activity was eluted with a linear gradient of ammonium sulfate at about 0.2 M, and fractions with the enzyme activity was collected as purified enzyme preparation. The amount of enzyme activity, specific activity and yield of the  $\alpha$ -isomaltosyl glucosaccharide-forming enzyme in each purification step are in Table 1.

**Table 1**

Enzyme	Amount of Enzyme (units/g sub.)	Content (%)		
		AA-2G	AA-5G	AA-6G
IMG* from <i>Arthrobacter</i> sp.	5	22.5	0.0	0.0
	10	24.0	0.0	0.0
	20	24.3	0.0	0.0
CGTase** (Control)	300	25.5	0.8	0.3

\* IMG;  $\alpha$ -isomaltosyl glucosaccharide-forming enzyme

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**\*\*CGTase; cyclomaltodextrin glucanotransferase**

**Please replace the paragraph beginning on page 23, line 3, with the following amended paragraph:**

A test for investigating whether the following each saccharide can be used or not as a glucosyl donor for transferring glucosyl residue to L-ascorbic acid by  $\alpha$ -isomaltosyl glucosaccharide-forming enzyme was carried out. A solution containing glucose, maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose, maltoheptaose, isomaltose, isomaltotriose, isopanose, trehalose, kojibiose, nigerose, neotrehalose, cellobiose, gentiobiose, maltitol, maltotriitol, lactose, sucrose, erlose, selaginose, maltosylglucoside, isomaltosylglucoside,  $\alpha$ -cyclodextrin,  $\beta$ -cyclodextrin,  $\gamma$ -cyclodextrin, amylose, amylopectin, glycogen, pullulan, or dextran was prepared. To each solution, L-ascorbic acid was further added and the concentrations of the saccharide and L-ascorbic acid were adjusted to 2% (w/v). To each substrate solution, purified  $\alpha$ -isomaltosyl glucosaccharide-forming enzyme preparation, obtained by the method in Experiment2, was added to give 3 units/g-substrate of the amount of enzyme, and the concentration of the substrate was adjusted to 1.6% (w/v), and followed the enzyme reaction at 40°C, pH 6.0 for 20 hours. The formation of AA-2G in the reaction mixture was detected by thin-layer chromatography (abbreviated as "TLC", hereinafter) using a silica gel plate. A mixture of n-butanol, pyridine, and water (volume ratio of 6:4:1) was used as a solvent for the development. After developing samples once on "KIESELGEL 60F<sub>254</sub>", silica gel-aluminum plate (20 x 20 cm) commercialized by Merck Ltd. Japan, Tokyo, Japan, AA-2G and L-ascorbic acid were detected by irradiating ultraviolet-ray. From the results of TLC analysis, the formation of AA-2G was evaluated. The results are in Table 23.

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IMG*	CGTase**	Content (%)		
		AA-2G	AA-5G	AA-6G
10	0	25.1	0.0	0.0
10	1	29.3	0.0	0.0
10	2	30.1	0.0	0.0
10	5	30.8	0.1	0.0
10	10	30.9	0.1	0.0
10	100	30.6	0.8	0.3
0	1	4.3	0.0	0.0
0	2	5.4	0.0	0.0
0	5	7.8	0.2	0.0
0	10	10.4	0.3	0.1
0	100	29.5	0.9	0.3

\* IMG;  $\alpha$ -isomaltosyl glucosaccharide forming enzyme

\*\*CGTase; cyclomaltdextrin glucanotransferase

**Please replace the paragraph beginning at page 24, line 5, with the following amended paragraph:**

As is evident from the results in Table 23, it was revealed that  $\alpha$ -isomaltosyl glucosaccharide-forming enzyme formed AA-2G by using saccharides, having a maltose structure at their non-reducing end and the glucose polymerization degree of three or higher as glucosyl donors and transferring a glucosyl residue to L-ascorbic acid. Further, it was also revealed that  $\alpha$ -isomaltosyl glucosaccharide-forming enzyme formed AA-2G by using saccharides, having a glucose polymerization degree of two such as maltose, kojibiose, nigerose, neotrehalose, maltotriitol, erlose as glucosyl donors.

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**Please replace the paragraph beginning at page 25, line 19, with the following amended paragraph:**

The contents of L-ascorbic acid and the transglucosylation products from L-ascorbic acid, d.s.b., were determined by measuring those absorbance at 238 nm using "UV-8020", a spectrophotometer commercialized by Tosoh Corporation, Tokyo, Japan; and measuring the composition of the reaction mixture including those using "RI-8020", a refractive index detector commercialized by Tosoh Corporation, Tokyo, Japan. The results are in Table 31.

**Please replace the paragraph beginning at page 26, line 1, with the following amended paragraph:**

Table 3 Table 1

Glucoamylase treatment	Content (%)		
	AA-2G	AA-2Gn*	L-Ascorbic acid
before	18.7	0.4	40.3
after	19.1	0.0	40.3

\*AA-2Gn means derivatives of AA-2G, having a structure of binding one or more glucopyranosyl residues to AA-2G.

As is evident from the results in Table 31, AA-2G in a content of about 18.7% and a transglucosylation product not identical with AA-2G in a content of about 0.4% were formed as the transglucosylation products by  $\alpha$ -isomaltosyl glucosaccharide-forming enzyme. After the glucoamylase treatment, it was revealed that the transglucosylation product not identical with AA-2G was disappeared to give AA-2G as a sole

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transglucosylation product from L-ascorbic acid and the content of AA-2G was increased. Taking account of the action pattern of glucoamylase, it was considered that the transglucosylation product not identical with AA-2G is glycosyl L-ascorbic acid constructed by binding one or more glucopyranosyl-residues with AA-2G; and that the glycosyl-residue constructed by one or more glucopyranosyl residues was hydrolyzed by glucoamylase to convert the glycosyl L-ascorbic acid into AA-2G.

**Please replace the paragraph beginning at page 27, line 29, with the following amended paragraph:**

An aqueous substrate solution containing 5% of L-ascorbic acid, 5% of "PINEDEX #1", a partial starch hydrolyzate commercialized by Matsutani Chemical Industry Co., Ltd., Osaka, Japan, and 1 mM calcium chloride was adjusted to pH 5.0. To the substrate solution, the purified  $\alpha$ -isomaltosyl glucosaccharide-forming enzyme prepared by the method of Experiment 2 was added to give the amount of enzyme of 5 to 20 units/g-partial starch hydrolyzate, and followed the enzyme reaction at 50°C for 48 hours. After the completion of the reaction, each reaction mixture was boiled at 100°C for 10 min to inactivate enzyme, cooled to 40°C, admixed with 40 units/g-partial starch hydrolyzate of glucoamylase commercialized by Seikagaku Corporation, Tokyo, Japan, and followed the enzyme reaction at 40°C for 16 hours. The contents, d.s.b., of AA-2G, AA-5G, and AA-6G of each reaction mixture were determined by HPLC described in Experiment 5 by using a commercially available AA-2G commercialized by Hayashibara Biochemical Laboratories Inc., Okayama, Japan; and AA-5G and AA-6G, prepared by the method described in the specification of Japanese Patent No. 3,134,235, as standards. Except for using 300 units/ g-partial starch hydrolyzate of CGTase

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commercialized by Hayashibara Biochemical Laboratories Inc., Okayama, Japan, as a substitute of  $\alpha$ -isomaltosyl glucosaccharide-forming enzyme, the same reaction was carried out and the contents of AA-2G, AA-5G and AA-6G were determined. The results are in Table 42.

**Please replace the paragraph beginning at page 29, line 1, with the following amended paragraph:**

Table 4-Table 2

Enzyme	Amount of Enzyme (units/g-sub.)	Content (%)		
		AA-2G	AA-5G	AA-6G
IMG* from <i>Arthrobacter</i> sp.	5	22.5	0.0	0.0
	10	24.0	0.0	0.0
	20	24.3	0.0	0.0
CGTase** (Control)	300	25.5	0.8	0.3

\* IMG;  $\alpha$ -isomaltosyl glucosaccharide-forming enzyme

\*\*CGTase; cyclomaltodextrin glucanotransferase

As is evident from the results in Table 42,  $\alpha$ -isomaltosyl glucosaccharide-forming enzyme formed AA-2G as a sole transglucosylation product and not formed AA-5G and AA-6G. The control enzyme, CGTase, formed AA-2G in almost same level with the case of  $\alpha$ -isomaltosyl glucosaccharide-forming enzyme and also formed AA-5G and AA-6G to give the contents of 0.8% and 0.3%, respectively.

**Please replace the paragraph beginning at page 29, line 21, with the following amended paragraph:**

An aqueous substrate solution containing 5% of L-ascorbic acid, 21% of "PINEDEX #1", a partial starch hydrolyzate commercialized by Matsutani Chemical Industry Co., Ltd., Osaka, Japan, and 1 mM calcium chloride was adjusted to pH 5.0. To the substrate solution, 10 units/g-partial starch hydrolyzate of the purified  $\alpha$ -isomaltosyl glucosaccharide-forming enzyme prepared by the method of Experiment 2 and 1 to 100 units/g-partial starch hydrolyzate of CGTase were added, and followed the enzyme reaction at 50°C for 24 hours. After the completion of the reaction, each reaction mixture was boiled at 100°C for 10 min to inactivate enzyme, cooled to 40°C, admixed with 40 units/g-partial starch hydrolyzate of glucoamylase commercialized by Seikagaku Corporation, Tokyo, Japan, and followed the enzyme reaction at 40°C for 16 hours. The contents, d.s.b., of AA-2G, AA-5G, and AA-6G of each reaction mixture were determined by HPLC described in Experiment 5. Except for using  $\alpha$ -isomaltosyl glucosaccharide-forming enzyme alone or CGTase alone as control tests, the same transferring reaction was carried out and analyzed with the same manner. The results are in Table 53.

**Please replace the paragraph beginning at page 31, line 1, with the following amended paragraph:**

Table 5 Table 3

IMG* (units/g-sub.)	CGTase** (units/g-sub.)	Content (%)		
		AA-2G	AA-5G	AA-6G
10	0	25.1	0.0	0.0

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10	1	29.3	0.0	0.0
10	2	30.1	0.0	0.0
10	5	30.8	0.1	0.0
10	10	30.9	0.1	0.0
10	100	30.6	0.8	0.3
0	1	4.3	0.0	0.0
0	2	5.4	0.0	0.0
0	5	7.8	0.2	0.0
0	10	10.4	0.3	0.1
0	100	29.5	0.9	0.3

\* IMG;  $\alpha$ -isomaltosyl glucosaccharide-forming enzyme

\*\*CGTase; cyclomaltodextrin glucanotransferase

As is evident from the results in Table 53, in the case of using  $\alpha$ -isomaltosyl glucosaccharide-forming enzyme alone, AA-2G was formed in a content of about 25% as a sole transglucosylation product from L-ascorbic acid, and AA-5G and AA-6G were not formed. In the case of using both  $\alpha$ -isomaltosyl glucosaccharide-forming enzyme and CGTase, the content of AA-2G was increased to about 29 to 31% and the formation of AA-5G and AA-6G were as follows:

1 to 2 units/g of CGTase; AA-5G and AA-6G were not formed.

5 to 10 units/g of CGTase; AA-5G in the content of about 0.1% was formed.

100 units/g of CGTase; AA-5G and AA-6G was formed in the contents of about 0.8% and 0.1%, respectively.